Journal of Wildlife Diseases, 46(1), 2010, pp. 283–286

Experimental Infection of Liver Flukes, *Fasciola hepatica* and *Fascioloides magna*, in Bison (*Bison bison*)

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ABSTRACT: This experimental study was conducted to evaluate the susceptibility of American bison (*Bison bison*) to liver flukes, *Fascioloides magna* and *Fasciola hepatica*. Six bison were each experimentally inoculated with 600 metacercariae of *Fascioloides magna*, and three were later treated with triclabendazole suspension at 40 mg/kg of body weight. Four additional bison were each experimentally inoculated with 600 metacercariae of *Fasciola hepatica*. Five control bison were placebo controls. Two controls and all inoculated bison were euthanized 10 mo (*Fascioloides magna*) and 7 mo (*Fasciola hepatica*) after inoculation. None of the control bison or the bison inoculated with *Fascioloides magna* had flukes or lesions characteristic of fluke infection at necropsy. All four bison inoculated with *Fasciola hepatica* had characteristic liver fluke lesions at necropsy, and three of four bison contained four, 103, and 111 adult flukes, respectively. Fluke eggs were detected in feces of all *Fasciola hepatica*–inoculated bison during the experiment, but not from the *Fascioloides magna*–infected bison or control bison. Clinical signs of infection were not observed during the experiment, but hemoglobin and packed cell volumes were lower in the *Fasciola hepatica* bison when compared to controls, and eosinophil levels were increased. Triclabendazole at 40 mg/kg of body weight appeared to be safe in bison because no toxic reactions were observed. Results from this study indicated bison are susceptible to infection with *Fasciola hepatica* and are efficient definitive hosts. Because no *Fascioloides magna* were recovered, bison may have a decreased susceptibility or innate resistance to *Fascioloides magna* infection, which may account for a lack of reported infections in this host.

Key words: Bison, *Bison bison*, experimental study, *Fasciola hepatica*, *Fascioloides magna*, liver flukes.

Fasciola hepatica and *Fascioloides magna* are two relatively common liver flukes that infect domestic and wild animals throughout parts of North America (Pybus, 2001). *Fascioloides magna* inhabits the parenchyma of the liver and is found primarily in the normal definitive hosts, white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elephus canadensis*), in which limited pathogenicity occurs. In aberrant hosts such as moose (*Alces alces*) domestic goats, domestic sheep, llamas (*Lama glama*), cattle and some other species, the life cycle is usually not completed (Pybus, 2001) and some of the hosts such as sheep and goats routinely die from infection (Foreyt, 1976; Foreyt and Leathers, 1980). Recently, *Fascioloides magna* infections were considered to be the greatest single source of mortality in a declining moose population in northwestern Minnesota (Murray et al., 2006). To our knowledge, there have been no documented reports of *Fascioloides magna* in bison (*Bison bison*) in over 50 yr. However, in 1924, *Fascioloides magna* was reported from two of an unspecified number of bison examined from Buffalo Park at Wainwright, Alberta, Canada (Cameron, 1924). *Fasciola hepatica* has a wide host range throughout much of the world. In North America, it is commonly found in domestic ruminants, but is uncommon in wild ruminants (Pybus, 2001). In bison, *Fasciola hepatica* has been reported from one bison in Montana, USA, (Locker, 1953) and one bison in Wyoming, USA (Bergstrom, 1967). The American bison is a native ruminant of North America and is a host of numerous parasites that also infect a variety of other wild and domestic ruminants. Because liver flukes have only rarely been reported

from bison, the purpose of this paper was to determine the susceptibility of bison to *Fasciola hepatica* and *Fascioloides magna* given known numbers of infective metacercariae and to document potential pathogenicity. These studies were conducted at the Idaho Department of Fish and Wildlife Health Laboratory (near Caldwell, Idaho, USA, 43°60′N, 116°50′W) between September 2001 and July 2002. All bison in these experiments were born in captivity, but the original parent stock was from Yellowstone National Park, Wyoming, USA. Bison were maintained in large outdoor pens and were fed alfalfa hay. Fresh water and mineral salt were available at all times.

Nine 4-yr-old bison bulls were used in the *Fascioloides magna* experiment. On 6 September 2001, each of six bison were inoculated with 600 *Fascioloides magna* metacercariae, and three were placebo controls. Mean weight of the bison was 680 kg (range 611–821). Metacercariae were obtained from Baldwin Enterprises (Monmouth, Oregon, USA), and were originally of white-tailed deer origin. Viability of the metacercariae was determined by movement of the flame cells, and metacercariae were counted under a dissecting microscope (30×) the evening before inoculation and stored in distilled water overnight at 4 C. On the day of inoculation, metacercariae were transferred in water to a gelatin capsule, and given orally using a balling gun (Nasco West, Modesto, California, USA). The three noninoculated control bison were given an equal amount of water in a gelatin capsule using a balling gun. On 11 December 2001 (approximately 12 wk after inoculation), three of six inoculated bison were treated orally with a 10% suspension of triclabendazole (Fasinex, Novartis Animal Health, Inc., Basel, Switzerland) at 40 mg/kg of body weight using a drenching syringe. Fecal samples were collected monthly throughout the experiment from each animal either directly from the rectum or from the ground immediately after defecation. Fecal samples were analyzed for gastrointestinal parasites, lungworms, and flukes using a sugar flotation technique, Baermann technique, and modified sedimentation technique, respectively, as described by Foreyt (2001). Blood samples in ethylenediaminetetraacetic acid (EDTA) were collected from the jugular vein of all bison 6 mo after inoculation, and a complete blood count (CBC) was done. Approximately 10 mo after inoculation and 7 mo after treatment, all six inoculated bison were euthanized with a captive bolt at a commercial slaughter facility on 17 July 2002. The three control bison were not euthanized.

For the *Fasciola hepatica* experiment, on 20 December 2001, four bison were each inoculated with 600 metacercariae of *Fasciola hepatica* of cattle origin. Source of the metacercariae, methods of inoculation, bison management, and data collection were the same as described for the *Fascioloides magna* experiment. Two placebo control bison were given water in gelatin capsules on the same day the other four bison were infected. The inoculated bison included three 2-yr-old females and one 1-yr-old male with a mean weight of 379 kg (range 305–423). The two controls were 2-yr-old female bison. Blood samples in EDTA were collected from the jugular vein of all animals 15 wk after inoculation, and a CBC was done. All six bison were euthanized at the same time as the other bison, approximately 7 mo after inoculation.

At necropsy, none of the six bison inoculated with *Fascioloides magna* were infected (Table 1), and lesions compatible with *Fascioloides magna* infection including hemorrhage, black pigment, fibrosis, etc. were not detected. Fluke eggs or lungworm larvae were not recovered from feces from inoculated or control bison during the experimental period. Other parasite eggs detected in low numbers included <30 strongyle eggs per gram of feces, which were recovered from two

TABLE 1. Numbers of liver flukes recovered from experimentally inoculated bison.

Bison no.[a]	Number of *Fasciola hepatica* recovered	Bison no.[b]	Number of *Fascioloides magna* recovered	Bison no.[c]	Number of *Fascioloides magna* recovered
R-17	11	B-49	0	B1-44	0
B-20	103	W-99	0	B1-36	0
R-84	4	B1-36	0	B1-28	0
B1-22	Lesions only				

[a] Inoculated with 600 *Fasciola hepatica* metacercaria.

[b] Inoculated with 600 *Fascioloides magna* metacercariae and treated with triclabendazole.

[c] Inoculated with 600 *Fascioloides magna* metacercariae and left untreated.

treated and two control bison. Blood values for all inoculated bison were within normal published values (Marler, 1975), and were similar to the control bison. Mean hemaglobin (Hb), packed cell volume (PCV), and eosinophil levels were 18.6 (range 17.1–20.4), 55.3% (range 51–62), and 4% (range 0–8), respectively.

Three of the four bison inoculated with *Fasciola hepatica* had four, 103, and 111 mature flukes at necropsy (Table 1). Representative samples of *Fasciola hepatica* (US National Parasite Collection [USNPC] 101611.00) were deposited in the USPNC (Agricultural Research Service, US Department of Agriculture, Beltsville, Maryland, USA).The inoculated bison in which flukes were not recovered (Bl-22) had hyperplasic bile ducts characteristic of *Fasciola hepatica* infection, but flukes were not detected. Fluke eggs were detected in feces from all inoculated bison approximately 12 wk after inoculation and were present throughout most of the experimental period. Flukes or fluke eggs were not recovered from the two control bison. Mean Hb (16.7 g/100ml) and PCV (50%) values were lower in the inoculated bison than in the controls (Hb=18.6 g/100ml; PCV=55%), but were within normal values. Mean eosinophil levels were elevated in the inoculated bison (28%, range 13–52) compared with the control (0%), and were well above a normal mean of 7% (Marler, 1975).

The number of captive and free-ranging bison in North America in 2003 has been estimated by the National Bison Association (Denver, Colorado, USA) at over 350,000. Although numerous parasites have been reported from bison, liver flukes are relatively uncommon (Plumb et al., 1992). The six bison inoculated with *Fascioloides magna* (three treated and three untreated) did not have flukes or fluke lesions at necropsy, which indicated *Fascioloides magna* did not develop in these animals. Although *Fascioloides magna* is enzootic throughout many areas in North America (Pybus, 2001), to our knowledge, there have been no documented reports of *Fascioloides magna* in bison since 1923 (Cameron, 1924). It is therefore likely that bison have a reduced susceptibility or are innately resistant to infection with *Fascioloides magna*, which would account for their rare occurrence in bison. A second unlikely possibility is the metacercariae lost their viability during the 18 hr between observing them as live metacercariae and inoculation.

There is a dramatic difference among hosts regarding responses to infection with *Fascioloides magna*. In white-tailed deer and elk, *Fascioloides magna* matures and becomes entrapped in fibrous capsules within the liver parenchyma with minimal pathogenicity in their host. Eggs are released into the biliary system and passed in feces to complete the cycle in an appropriate snail intermediate host. In other animals, such as sheep and goats, *Fascioloides magna* infections usually kill the host because of excessive hepatic damage from migration before the fluke matures. In llamas and cattle, the flukes

often mature in the liver, but the eggs are not passed in the feces (Pybus, 2001). Infections in horses, primates, carnivores, and several other animals have not been reported because some hosts appear to be resistant to infection (Foreyt, 1979; Pybus, 2001). Additional experimental infections in bison and case history reports will eventually support or reject the hypothesis that bison are resistant hosts.

Based on our results, bison are highly susceptible hosts for *Fasciola hepatica* because all inoculated bison became infected, and over 100 flukes were recovered from two of the four. Therefore, evaluation for *Fasciola hepatica* infection using the fecal sedimentation technique or serologic tests should be accomplished in enzootic areas where bison are copastured in the vicinity of infected cattle, sheep, or goats, or when bison have anemia or eosinophilia.

We would like to thank J. Mulholland, M. Hoagland, and P. Mamer for their excellent assistance with the bison handling and the data collection.

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Received for publication 9 October 2008.